

KOUZARIDES -- Serial No.: 09/700,417

1. (Amended) An assay method for an agent which affects E2F acetylation, the method including:

- E
- C1
- (a) treating an acetylated E2F polypeptide or an acetylated E2F peptide with a test compound, or
  - (b) treating with a test compound an E2F polypeptide or an acetylated E2F peptide which comprises one or more lysine residues corresponding to those found at positions 117, 120 and 125 in wild-type E2F1, in which polypeptide or peptide one or more of said lysines is not acetylated, or
  - (c) bringing into contact a substance which includes a P/CAF polypeptide which acetylates E2F, a substance which includes an E2F polypeptide or an E2F peptide including a site acetylated by P/CAF, and a test compound;
- and, following step a, b or c,
- (d) determining acetylation of the E2F polypeptide or E2F peptide.

E

C2

3. (Amended) An assay method for an agent which affects E2F activity, the method comprising:

- (a) providing an E2F polypeptide which activates transcription from a promoter including an E2F binding site, a test compound, and a reporter construct including a promoter which includes an E2F binding site and which is operably linked to a reporter sequence for transcription

KOUZARIDES -- Serial No.: 09/700,417

thereof, under conditions wherein, in the absence of the test compound being an inhibitor of E2F acetylation, the reporter sequence is transcribed, or

E 1  
C2  
wt  
(b) providing an E2F polypeptide which activates transcription from a promoter including an E2F binding site, which polypeptide comprises one or more lysine residues corresponding to those found at positions 117, 120 and 125 in wild-type E2F1, and in which polypeptide or peptide one or more of said lysines is not acetylated, a test compound, and a reporter construct including a promoter which includes an E2F binding site and which is operably linked to a reporter sequence for transcription thereof, under conditions wherein if the test compound promotes acetylation of E2F the reporter sequence is transcribed, or

(c) providing an E2F polypeptide which interacts with P/CAF and activates transcription from a promoter including an E2F binding site, a P/CAF polypeptide which interacts with E2F, a test compound, and a reporter construct including a promoter which includes an E2F binding site and which is operably linked to a reporter sequence for transcription thereof, under conditions wherein, in the absence of the test compound being an inhibitor of

KOUZARIDES -- Serial No.: 09/700,417

interaction between P/CAF and E2F, the reporter sequence is transcribed;

and, following step a, b or c

(d) determining promoter activity.

E (

4. (Amended) An assay method for an agent which modulates interaction between P/CAF and E2F, the method including:

C2

(a) bringing into contact a first substance including a P/CAF polypeptide or a P/CAF peptide, a second substance including an E2F polypeptide or an E2F peptide, and a test compound under conditions in which, if the test compound does not disrupt the interaction between P/CAF and E2F, the first and second substances interact; and

(b) determining interaction between the first and second substances.

5. An assay method for an agent which affects one or more of (i) ability of E2F to stimulate transcription, (ii) induction of S-phase in cells, (iii) oncogenicity of cells, and (iv) induction of apoptosis in cells, the method comprising:

(a) bringing into contact P/CAF and a test compound, and

KOUZARIDES -- Serial No.: 09/700,417

(b) determining P/CAF acetyltransferase activity;  
wherein a test compound which inhibits P/CAF  
acetyltransferase activity is identified as a candidate  
said agent.

E1  
C3  
8. (Twice Amended) A method according to claim 5  
wherein a test compound which inhibits P/CAF  
acetyltransferase activity is further tested for ability to  
affect one or more of (i) ability of E2F to stimulate  
transcription, (ii) induction of S-phase in cells, (iii)  
oncogenicity of cells, and (iv) induction of apoptosis in  
cells.

E1  
C4  
9. (Amended) An assay method for an agent which  
interacts with a region of P/CAF or a region of E2F, which  
region of P/CAF interacts with E2F and which region of E2F  
interacts with P/CAF, a said agent which interacts with a  
said region being a candidate modulator of interaction  
between P/CAF and E2F, the method including:

(a) bringing into contact a substance which includes  
a P/CAF peptide which interacts with E2F, or which includes  
an E2F peptide which interacts with P/CAF, and a test  
compound; and

E1  
C4  
cr  
KOUZARIDES -- Serial No.: 09/700,417

(b) determining interaction between said substance and the test compound.

C5  
12. (Twice Amended) A method according to claim 1 further comprising providing a said agent, or, where said agent is peptidyl, providing nucleic acid encoding a said agent, to cells to modulate one or more of (i) ability of E2F to stimulate transcription in the cells, (ii) induction of S-phase in the cells, (iii) oncogenicity of the cells, and (iv) induction of apoptosis in the cells.

Cancel claims 14-18 and add the following new claims in lieu thereof.

C6  
24. (New) A peptide fragment of E2F or of P/CAF, which peptide is about 40 amino acids or less and comprises one or more lysine residues corresponding to those found at positions 117, 120 and 125 in wild-type E2F1, and which modulates acetylation of E2F by P/CAF. E2F1  
para 13

25. (New) A peptide according to claim 24 which is about 20 amino acids in length.